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Radiation and hydrogen peroxide induced free radical damage to DNA

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In a previous publication (Ward *et al.*, 1985) we examined the production of intracellular DNA damage of the strand break type produced by hydrogen peroxide treatment of mammalian cells. To summarize our findings: V79 and HeLa cells when treated with hydrogen peroxide at 0°C are not readily killed – a treatment with 40 mM for 10 min is necessary to kill 63% (Figure 1). However the DNA of these cells showed significant levels of single strand breaks (SSB) at micromolar levels of the agent (50 micromolar for 10 min produced 5 Gray equivalents of damage: 5,000 SSB per cell (Elkind & Redpath, 1977)). This means that, if the yield of SSB produced by hydrogen peroxide is linearly dependent on concentration, 0.4 million SSB are produced per cell per lethal event. In contrast, for ionizing radiation the yield of SSB per cell per lethal event is 1,000 (Elkind & Redpath, 1977).

By the use of OH radical scavengers we showed that the species from hydrogen peroxide causing the damage was the OH radical and that the distance travelled by this species prior to reacting with DNA was on average 15 A. This distance is in agreement with the mechanism proposed for the production of OH radicals and their subsequent reaction with DNA: Hydrogen peroxide reacting in a Fenton reaction with a variable valency metal ion bound to the DNA.

Subsequent studies brought to light an anomaly: if the cells were treated at room temperature (24°C) or at 37°C, they were killed with much lower concentrations of hydrogen peroxide (Figure 1) ($D_o = 35$ micromolar) (Hofmann *et al.*, 1984; Ward *et al.*, 1985). We attempted to measure the yield of SSB after room temperature treatment. None (less than 100 per cell) were found at concentrations of hydrogen peroxide up to 10 mM. We rationalised that this was due to the rapid enzymatic repair of OH radical induced DNA SSB, which we have determined to have a half life of 4 min (Ward *et al.*, 1983*a*). In the previous communication (Ward *et al.*, 1985) we suggested that hydrogen peroxide killing at higher temperature (24°–37°C) was caused by the induction of DNA double strand breaks (DSB). The number of double strand breaks per cell necessary for cell kill has been shown to be low: for ionizing radiation only 40 DSB per cell are required for kill. The sensitivity of the neutral elution assay of DSB would not permit detection of such low levels of DSB, however even using the more sensitive SSB assay this yield could not be detected. The suggested mechanism of production of a DSB by hydrogen peroxide involves the initial production of the first SSB following a Fenton reaction (1) and (2):

$$M^+ + H_2O_2 \rightarrow M^{++} + OH \quad (1)$$

$$DNA + OH \rightarrow SSB \quad (2)$$

where M is a variable valency metal ion bound to a specific site in DNA. This was suggested to be followed by metabolic reduction of the resulting oxidised DNA-bound metal ion (3):

$$M^{++} \rightarrow M^+ \quad (3)$$

Subsequently a second molecule of hydrogen peroxide reacts at the same local site as the first again producing an OH radical (4):

$$M^+ + H_2O_2 \rightarrow M^{++} + OH \quad (4)$$

Reaction of the second OH radical with the intact DNA strand would then have the possibility of causing a DSB:

$$DNA\ SSB + OH \rightarrow DNA\ DSB \quad (5)$$

However, since we failed to find any SSB present (possibly due to rapid repair), we attempted to prevent the repair process and thus 'trap' any DSB produced. To do this the hydrogen peroxide treatment of cells was performed in the presence of either hypo- or hypertonic salt. Under these conditions repair of DNA DSB is effectively totally inhibited (Ward, 1986; Ward *et al.*, 1983*b*; Hinchcliff & McNally, 1986). However even in the presence of effective inhibition of DNA DSB repair, DSB were only observable at very high hydrogen peroxide concentrations (1 M hydrogen peroxide for 10 min produced 30 Gray equivalent DSB). It is possible that these anisotonic salt conditions affected the efficiency of the Fenton reaction – by dissociating the variable valency metal ion from the DNA, and/or by altering chromatin structure (Raaphorst *et al.*, 1978) in such a way as to limit access of hydrogen peroxide. Since in our experience the rate or repair of DNA DSB is much slower (half life 40 min (Evans *et al.*, 1986)) than that of SSB, it ought to be possible to measure the yield of DSB after brief exposures to hydrogen peroxide even in isotonic conditions. Therefore we have attempted to measure the yield of DNA DSB in cells exposed to varying concentrations of hydrogen peroxide for a shorter time period (10 min) i.e. so that initial yields of DNA DSB could be approached without the occurrence of significant repair. Again DNA DSB were observed only after treatment with 1 M hydrogen peroxide in a yield equivalent to 30 Gray. The

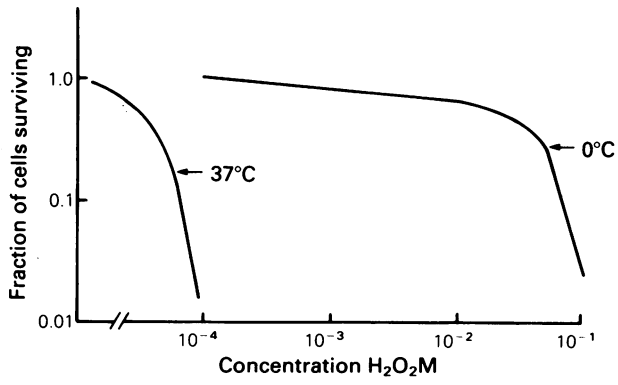

Figure 1 Survival curves for Chinese hamster V79–171 cells treated with hydrogen peroxide at 0°C or at 37°C. Cells were treated in suspension for 30 min at the indicated temperature. Then they were washed free of H_2O_2, plated out and incubated at 37°C for assay of survival.

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DSB assay is not sensitive at break yields equivalent to those necessary to cause cell killing, however if we assume that the yield of DSB is linearly dependent on hydrogen peroxide concentration, then, at the concentrations of hydrogen peroxide that cause cell killing, DNA DSB equivalent to about 1 CentiGray would be produced (only 0.4 per cell). One interpretation of this result is that hydrogen peroxide at room temperature does not kill cells by producing strand break damage to their DNA.

A linear extrapolation with concentration must be considered simplistic if the total reaction schema suggested for hydrogen peroxide production of DNA DSB is considered (1–5). It seems that the process should be at least dependent on the square of the hydrogen peroxide concentration. Of course other steps would be significant: The repair of SSB formed in step 2, which we have shown to be have a half life of 4 min, would be in competition with steps 3 and 4, thus reducing the probability of DNA DSB formation in any significant yields at micromolar peroxide concentrations.

A complication of this interpretation is that the number of variable valency metal ions bound to cellular DNA may be limiting. In the previous work we measured yield of DNA SSB only up to levels of 30,000 per cell. If this was an indication of the total number of bound metal ions (although the yield concentration curve did not show the approach to a limit) then this would be the theoretical maximum yield of DNA DSB. This maximum would be equivalent to 750 Gray of ionizing radiation, well within the sensitivity of the measurement technique. Thus we are left with an anomaly: How does hydrogen peroxide kill cells at 24°–37°C?

Molecular damage

Studies of the damage produced in DNA by hydrogen peroxide have a long history. In Table I the types of damage found after hydrogen peroxide treatment are summarized. Damage types produced include: strand breaks, base damage, base release and cross-links. Rhaese and Freese (1968*a*) observed that the treatment of DNA in solution with hydrogen peroxide leads to the release of undamaged bases as a result of oxidation of the deoxyribose at the 1′ position. They confirmed the mechanisms by using deoxynucleotide model systems (Rhaese & Freese, 1968*b*). The release of free bases following the attack of OH radicals generated by ionizing radiation has also been shown (Ward and Kuo, 1976). Massie *et al.* (1972) also measured base destruction and detected DNA interstrand DNA cross-links (CL). The latter type of damage was studied more fully by Lesko *et al.* (1982) who also observed DNA-protein CL in isolated chromatin treated with hydrogen peroxide.

It can be seen that the range of damage types produced by hydrogen peroxide treatment is the same as that seen for OH radicals produced by ionizing radiation (Ward, 1975). The yields of the various damage types after hydrogen peroxide treatment determined by different laboratories (Table I) may not be readily intercompared because of the different DNA preparations and treatment protocols used. The rates of damage production are dependent on the presence of uncontrolled amounts of impurity metal ion. However, Massie *et al.* (1972) did compare the yields of damage types in the same experimental set up. They found that the relative magnitudes of yields of the various types of damage are in the order base destruction > SSB > DSB > crosslinks, i.e. the same order as those for ionizing radiation.

It was recognized early that the mechanism by which hydrogen peroxide causes damage is via hydroxyl radicals, Schweitz (1969) showed that the rate of damage production – measured by DNA depolymerization – was greater in the presence of cuprous, ferrous or ferric ions. He discusses the relative efficiency of these ions in terms of their ability to bind to 'inner' and 'outer' sites in the DNA. A more recent description of the binding of ions to DNA is the ion condensation theory of Manning (1978) as developed by Le Bret and Zimm (1984). Here the tightness of binding of cations to DNA treated as an infinite polyanionic cylinder is considered.

In the work of Schweitz (1969) and of Massie *et al.* (1972) it was shown that the production of DNA DSB was non-linear with hydrogen peroxide concentration and with time of treatment. Thus it was suggested that such damage was formed as a result of coincident single strand breaks, each produced by a separate OH radical. Schweitz calculated the frequency of such an occurrence and found the calculated yield-dose relationship to be consistent with this proposed mechanism.

In those studies carried out in neutral aqueous solution the process by which the metal ion is converted to the necessary reduced state is not clear. One possible mechanism is via the slow process involving hydrogen peroxide:

$$Fe^{+++} + H_2O_2 \rightarrow Fe^{++} + H^+ + HO_2 \qquad (6)$$

Cell killing by radiation and hydrogen peroxide

The evidence is strong that ionizing radiation causes cell killing by the production of locally multiply damaged sites (LMDS) in DNA (for references see Ward, 1985). These are produced in regions of high radical density present immediately after the occurrence of the energy deposition events (Ward, 1985). As discussed above such lesions (if DSB are a measure of the overall yield of LMDS) are not apparent

Table I Hydrogen peroxide damage produced in DNA

Type of damage	*Treatment conditions*	*Yield*	*Reference**
Base release	005 M H_2O_2 10 μM Fe^{3+} 37°C 5 days	All bases equal 8 μM each	3
Depolymerization	0.04 M H_2O_2 100 μM Cu^{2+} 37°C 2 days	10 μM	4
	100 μM Fe^{3+}	0.4 μM	
Base damage	0.088 M H_2O_2 37°C 20 days	7.6 μM (total)	2
SSB		0.07 μM	
DSB		non-linear	
Crosslink		indirect measure	
DNA-DNA CL		not-determinable	1
DNA-protein CL (in chromatin)		not-determinable	

*1. S.A. Lesko (1972), *Biochim. Biophys. Acta*, **272**, 539.
2. H.A. Massie *et al.* (1982), *Biochemistry*, **21**, 5010.
3. H.-J. Rhaese & E. Freese (1968), *Biochim. Biophys. Acta*, **155**, 476.
4. H. Schweitz (1969), *Biopolymers*, **18**, 101.

after hydrogen peroxide treatment. However, it must be remembered that when cells are treated at ambient temperatures and above, repair processes compete with the damage production processes so that any measured yield at any specific time is the resultant of this competition. Considering this competition in the case of hydrogen peroxide treatment may provide an explanation for the anomaly described above: The initial reactions of hydrogen peroxide with the reduced metal ions will lead to the production of singly damaged sites, including base damage and SSB. The latter have a half-life of 4 min, while those of the former are longer. For thymine glycol type damage Mattern *et al.* (1975) measured a half life for removal from the DNA of 7 min after irradiation of WI 38 and CHO cells, and for removal of 8-hydroxy adenine we (West, West & Ward unpublished) measured a half life of 10 min for removal from the DNA of irradiated V79 cells. The half life of each of the large variety of base damage products may be different.

Subsequent to the formation of the first damaged site the metal ion oxidised in the production of the OH radical (reaction 1) must be reduced back (reaction 3) and then react with a second hydrogen peroxide molecule to produce a double damaged site (reactions 4 and 5). Therefore it is more likely that the second OH reacting will react with a site containing a base damaged site than a SSB (because of the longer half-life). The lethal damage from hydrogen peroxide at the higher temperatures may still be a doubly damaged site containing a damaged base – this would not have been detected in the DSB assay. Such LMDS have the potential to be as lethal as a DSB (Ward, 1985).

Previously we have described how the formation of a LMDS in DNA results in the irretrievable loss of genetic information, regardless of whether the damaged site is ligated together or remains as a break. Simplistically, cell death could be thought of as occurring as a result of production of this damage within the gene of a protein whose presence is necessary for cell survival. The fact that death does not occur till after several cell divisions may indicate that the essential protein is gradually diluted as cell division and probably proteolysis occur.

Comparison with other noxae

Ionizing radiation is an efficient cell killer in terms of numbers of damaged sites per genome per lethal event compared to many other agents. In Table II the effectiveness of a variety of agents in cell killing and production of DNA lesions are compared. Numbers of lesions per cell per lethal event are calculated for each agent. The product of the concentration and time of treatment necessary to kill 63% of the cells exposed is indicated for each agent. At this amount of kill there is, on average, one lethal event per cell. With information of numbers of damaged DNA sites as a function of time and concentration of treatment, the number of damaged sites per lethal event were calculated. In some instances it was necessary to assume a linear relationship between concentration times time and yield of damage.

It can be seen that there are two ranges of lethal events per damaged site. A low range exemplified by ionizing radiation and bleomycin, and a high range shown by hydrogen peroxide at 0°C, ultraviolet light and acetyl amino fluorene. It is realized that each agent causes a range of damage types, but the compartmentalization into two classes is striking. It is clear that agents which can cause DNA DSB or LMDS can kill with only 30–40 such damaged sites per genome. The other agents producing single damaged sites require orders of magnitude more lesions to have an effect on cell viability. It is unclear whether the lethal events at these high levels of damage are the result of coincident single events causing LMDS or to problems the cell has in attempting to repair large numbers of damaged sites.

Again it must be said that hydrogen peroxide at room temperature is an anomaly, we can not estimate the DNA damage produced. Peroxide at 0°C is toxic in the same range (lesions per cell) as other agents (UV light, AAF, etc.).

Restriction enzyme cutting

Since it appears that the DNA DSB is a major cause of cell killing, we have attempted to simulate this damage using restriction endonucleases. In this case we would not produce a range of damage types but only DNA double strand cuts (DSC). It should be pointed out that the cuts produced by the enzyme treatment are different from breaks produced by damaging agents in that there is no loss of information from the DNA. Therefore such cuts can be repaired by the action of DNA ligase with no possibility of residual damage. Of course if the cut is not ligated then problems can ensue.

Table II Yields of DNA damage necessary to kill 63% cells

Agent	D_{37} (*Conc.* × *Time*)	*DNA lesion*	*Number of lesions per cell per* D_{37}	*Reference**
Ionizing radiation	100 rad	SSB	1,000	1
		DSB	40	
Bleomycin A2	5.5 μg–1 h	SSB	150	2, 3
		DSB	30	
UV light	10 J m^{-1}	TT dimer	400,000	4, 5, 6
		SSB	100	
Hydrogen peroxide 0°C	40 mM–10 min	SSB	400,000	7
37°C	40 μM–1 h	?		
Acetylamino fluorene	1.7 μM–3 h	Adduct	700,000	8
Other similar aromatic amides behave equivalently.				9

*1. M.M. Elkind & J.L. Redpath (1977).
2. J.S. Lazo *et al.* (1985).
3. M.O. Bradley & K.W. Kohn (1979).
4. A.M. Rauth (1970).
5. R.B. Setlow *et al.* (1969).
6. J. Jagger (1976).
7. J.F. Ward *et al.* (1985).
8. R.H. Heflich *et al.* (1980).
9. V.M. Maher *et al.* (1981).

To introduce restriction enzymes into the cells we have used a permeabilisation protocol developed by Julian Preston (Personal Communication). Cells were treated with various levels of enzyme for 20 min. Subsequently the enzyme was removed by extensive washing and cell viability assayed. Permeabilisation in the absence of the restriction enzyme resulted in retention of >90% cell survival. As the enzyme concentration increased cell kill was observed and the plot of cell survival *versus* enzyme concentration is shown in Figure 2. The level necessary to produce 63% kill was 100 units Msp I per 400,000 cells. The amount of cell kill from the various enzymes differs. Hae III is the most efficient followed by Msp I and Pvu II then Hind III and Bam HI.

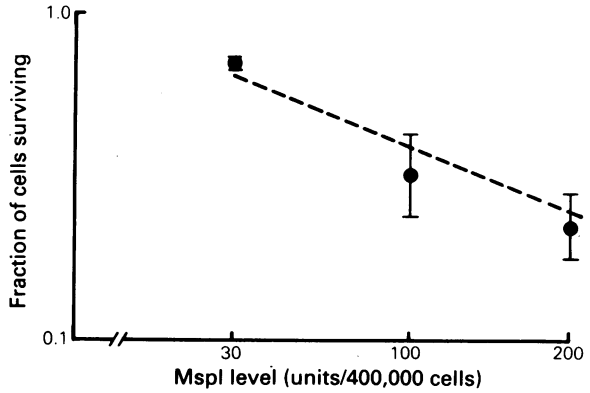

Figure 2 Survival curve for Chinese hamster V79–171 cells treated with restriction endonuclease MspI. Treatment was carried out with the cells attached to a 10 cm dish using the stated number of enzyme units dissolved in 0.1 ml of permeabilization buffer. Cells were treated for 20 min, washed throughly, trypsinized and replated for survival assay.

To determine the involvement of DNA DSC in cell killing we measured the yield of DNA DSB by neutral elution immediately after the end of the enzyme treatment prior to washing. Of course the amount of DSC produced represents the resultant of the number of cuts produced by restriction nicking and enzymatic rejoining. We have found by measuring DNA DSB that per unit, Msp I is the most effective cutter studied followed in order by Bam HI, Hae III, Pvu II, and Hind III. A comparison of this series with that of cell killing efficiency shows that the number of strand breaks is not the only determinant of cell killing. It is apparent that the blunt cutters Pvu II and Hae III are more efficient at killing, followed by the two cutter Msp I and lastly the four cutters.

This is in agreement with the findings of Bryant (1984) who found that treatment with the blunt end cutter Pvu II produced chromosome aberrations while the cohesive end cutter Bam HI did not.

We conclude from these findings that all DNA DSB are not equally lethal. Those which have the constituent single strand breaks directly opposite would be more lethal than those which are offset. This finding is in agreement with a prediction made in an earlier paper (Ward, 1985).

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References

BRADLEY, M.O. & KOHN, K.W. (1979). X-ray induced DNA double strand break production and repair in mammalian cells as measured by neutral filter elution. *Nucleic Acids Research*, **7**, 793.

BRYANT, P.E. (1984). Enzymatic restriction of mammalian DNA using Pvu II and Bam H1: Evidence for the double strand break origin of chromosomal aberrations. *Int. J. Radiat. Biol.*, **46**, 57.

ELKIND, M.M. & REDPATH, J.L. (1977). Molecular and cell biology of radiation lethality. In *Cancer, A Comprehensive Treatise*, Becker, F.F. (ed) **6**, p. 51. Plenum Press: New York.

EVANS, J.W., LIMOLI, C.L. & WARD, J.F. (1986). Does neutral elution measure intracellular levels of DNA double strand breaks (DSB). Abstract Cq17, *Proc. 34th Meeting Radiat. Res. Soc.*, Las Vegas.

HEFLICH, R.H., HAZARD, R.M., LOMMEL, L., SCRIBNER, J.D., MAHER, V.H. & McCORMICK, J.J. (1980). A comparison of the DNA binding, cytotoxicity and repair synthesis induced in human fibroblasts by reactive derivatives of aromatic amide carcinogens. *Chem.-Biol. Interactions*, **29**, 43.

HINCHCLIFF, M. & McNALLY, N.J. (1986). The effect of post-irradiation anisotonic treatment on the repair of DNA damage in V79 cells. Abstract Eo14, *Proc. 34th Meeting Radiat. Res. Soc.*, Las Vegas.

HOFFMAN, M.E., MELLO-FILHO, A.C. & MENEGHINI, R. (1984). Correlation between cytotoxic effect of hydrogen peroxide and the yield of DNA strand breaks in cells of different species. *Biochim. Biophys. Acta*, **781**, 234.

JAGGER, J. (1976). Ultraviolet inactivation of biological systems. In *Photochemistry and Photobiology of Nucleic Acids*, Wang, S.Y. (ed) **II**, Biology p. 147. Academic Press: New York.

LAZO, J.S., BRAUN, I.D., MEANDZIJA, B., KENNEDY, K.A., PHAM, E.T. & SMALDONE, L.F. (1985). Lidocaine potentiation of bleomycin A2 cytotoxicity and DNA strand breakage in L1210 and human A-253 cells. *Cancer Res.*, **45**, 2103.

LEBRET, M. & ZIMM, B.H. (1984). Monte Carlo determination of the distribution of ions about a cylindrical polyelectrolyte. *Biopolymers*, **23**, 271.

LESKO, S.A., DROCOURT, J.-L. & YANG, S.-U. (1982). Deoxyribonucleic acid-protein and deoxyribonucleic acid interstrand cross-links induced in isolated chromatin by hydrogen peroxide and ferrous ethylenediaminetetraacetate chelates. *Biochemistry*, **21**, 5010.

MAHER, V.M., HEFLICH, R.H. & McCORMACK, J.J. (1981). Repair of DNA damage induced in human fibroblasts by N-substituted aryl compounds. In *NCI* Monograph No. 58 *Carcinogenic and Mutagenic N-Substituted Aryl Compounds*, 217.

MANNING, G.S. (1978). The molecular theory of polyelectrolyte solutions with applications to the electrostatic properties of polynucleotides. *Quart. Rev. Biophys.*, **11**, 179.

MASSIE, H.R., SAMMIS, H.V. & BAIRD, M.B. (1972). The kinetics of degradation of DNA and RNA by H_2O_2, *Biochim. Biophys. Acta*, **272**, 539.

MATTERN, M.R., HARIHARAN, P.V. & CERUTTI, P.A. (1975). Selective excision of gamma ray damaged thymine from the DNA of cultured mammalian cells. *Biochim. Biophys. Acta*, **395**, 48.

RAAPHORST, G.P., LAW, P. & KRUUV, J. (1978). Water content and spin lattice relaxation times of cultured mammalian cells subjected to various salt, sucrose, or DMSO solutions. *Physiol. Chem. Physics*, **10**, 177.

RAUTH, A.M. (1970). Effects of UV light on mammalian cells in culture. *Curr. Topics Radiat. Res.*, **VI**, 195.

RHAESE, H.-J. & FREESE, E. (1986*a*). Chemical analysis of DNA alterations. I. Base liberation and backbone breakage of DNA and oligodeoxyadenylic acid induced by hydrogen peroxide and hydroxylamine. *Biochim. Biophys. Acta*, **155**, 476.

RHAESE, H.-J. & FREESE, E. (1968*b*). Chemical analysis of DNA alterations. II. Alteration and liberation of bases of deoxynucleotides and deoxynucleosides induced by hydrogen peroxide and hydroxylamine. *Biochim. Biophys. Acta*, **155**, 491.

SCHWEITZ, H. (1969). Degradation du DNA par H_2O_2 en presence d'ions Cu^{++}, Fe^{++}, et Fe^{+++}. *Biopolymers*, **8**, 101.

SETLOW, R.B., REGAN, J.D., GERMAN, J. & CARRIER, W.L. (1969). Evidence that xeroderma pigmentosum cells do not perform the first step in the repair of ultraviolet damage to their DNA. *Proc. Nat. Acad. Sci. USA*, **64**, 1035.

WARD, J.F. (1975). Molecular mechanisms of radiation-induced damage to nucleic acids. *Adv. Radiat. Biol.*, **5**, 182.

WARD, J.F. (1985). Biochemistry of DNA lesions. *Radiat. Res.*, **104**, S103.

WARD, J.F. (1986). Mechanisms of DNA repair and their potential modification for radiotherapy. *Int. J. Radiat. Oncol. Biol. Phys.*, **12**, 1027.

WARD, J.F., BLAKELY, W.F. & JONER, E.I. (1985). Mammalian cells are not killed by DNA single strand breaks caused by hydroxyl radicals from hydrogen peroxide. *Radiat. Res.*, **103**, 383.

WARD, J.F., BLAKELY, W.F. & MOBERLY, J.B. (1983). A comparison of the enzymatic repair kinetics of ionizing radiation and of hydrogen peroxide induced DNA strand breaks in V79 cells. *Radiat. Res.*, **94**, 629.

WARD, J.F., JONER, E.I. & BLAKELY, W.F. (1983). Effects of hypotonic salt treatment on DNA damage production and its repair in HeLa cells. Abstract B2-41, *Proc. 7th International Congress of Radiation Research*, Amsterdam.

WARD, J.F. & KUO, I. (1976). Strand breaks, base release and post-irradiation changes in DNA gamma irradiated in dilute O_2-saturated aqueous solution. *Radiat. Res.*, **66**, 485.

Discussion

Michael: Does hydrogen peroxide treatment lead to interphase death or mitotic death?

Ward: Mitotic death.

Brown: Does the age response function for hydrogen peroxide treatment mimic that for radiation?

Ward: Blakely (Bethesda) has reported that cell kill following 0°C treatment using 100 mM hydrogen peroxide showed an age response that is the inverse of the radiation age response: for peroxide treatment V79 cells are resistant during G1, G2 and M and sensitive during S.

Elkind: If hydrogen peroxide produces radiation-like damage it should be possible to demonstrate additivity of action at an intracellular level.

Ward: We have used the 0°C treatment where we can put large numbers of single strand breaks in the DNA (equivalent to the yield which would be produced by 200 Gray) and the survival curve of cells so treated was no different from that from untreated cells.

Elkind: Why do you look upon this treatment as something that is acting like ionizing radiation if the evidence is limited to the production of breaks in DNA?

Ward: Hydrogen peroxide does not produce damage like ionizing radiation, it produces hydroxyl radical damage in the form of single strand breaks. These are equivalent to radiation produced single strand breaks. It does not produce double strand breaks which are probably more important lesions.

Myers: In view of the discussion earlier about whether hydroxyl free radicals are responsible for major damage only if they are formed very close, where is the hydrogen peroxide in the cell and what is its distribution, what is its concentration around the DNA?

Ward: The numbers quoted were the extracellular concentration. What we measured was yields of breaks in the DNA. We have shown that the production of those breaks can be prevented by putting dimethyl sulphoxide or tertiary butyl alcohol in the cell suspension. The scavenging kinetics indicate that OH radicals do not travel more than 20–30 Angstroms, suggesting that hydrogen peroxide reacts with metal ions on the DNA to produce OH radicals which then react.

Denekamp: Why, in the questions for discussion, have you suggested that hydrogen peroxide could be responsible for the reverse dose rate effect in cell transformation by neutrons.

Ward: The reasons for this suggestion are as follows:

1. Superoxide appears to be a promoter of transformation (see Borek, this meeting).
2. If superoxide is effective it probably mediates its effect via hydrogen peroxide, since superoxide itself is relatively unreactive.
3. The molecular yield (G value) for hydrogen peroxide production from neutron irradiation is 2–3 times higher than that from gamma radiation.
4. Catalase has a high Km for hydrogen peroxide and will consequently destroy little of the hydrogen peroxide produced at such low concentrations.
5. At lower dose rates hydrogen peroxide is produced at an even lower steady state concentration.
6. When the low dose rate experiments were carried out the cells were irradiated in a T-flask filled with 100 ml of medium whereas at higher dose rates they were more conventionally irradiated in dishes with 10 ml of medium. Since the hydrogen peroxide is formed as a consequence of water radiolysis more will be produced per cell per dose in the low dose rate geometry.

Elkind: Would not the 10% serum present shield the cells from any reactive material produced outside the cell?

Ward: When we looked at cell kill we found that in 10% serum we needed about two times as much hydrogen peroxide as that necessary in phosphate buffered saline to produce the same amount of kill (at 24°C).

Michael: We have later a contribution from Dr Link which may shed some light on this situation.

Greenstock: Is anything known of the effect of peroxide on repair enzymes, polymerases, nucleases etc., and is there a non-linear concentration effect?

Ward: I have no information on this. The question arises from the observed need for high concentrations necessary for kill at 0°C.

Wallace: In the comparison of ionizing radiation with hydrogen peroxide I have the following comment. We found that in prokaryotes, *E. Coli* in particular, treating the cells with a very low concentration of hydrogen peroxide confers protection against a subsequent high dose of hydrogen peroxide. This protection is concomitant with the production of a whole series of stress proteins that has been associated with oxidative stress, some few of which cross react with heat shock proteins but which is a whole different system under different operon regulation. If you treat *E. Coli* with very low concentrations of hydrogen peroxide you can show an increased resistance conferred on X-irradiated phage when they are introduced into these cells. (This is the work of Bruce Demple, Harvard.) This effect is not large but there is some cross relationship between hydrogen peroxide and oxidative type damage and its regulation in bacterial systems to ionizing radiation.

Ward: In our work with hydrogen peroxide plus radiation cells were held at 0°C during and between the exposures so it is unlikely that this would occur.

Wallace: This is a different experiment: We induce a repair system with low treatment with hydrogen peroxide and this confers resistance to subsequent treatment with either hydrogen peroxide or radiation. This implicates the same repair system, or set of repair systems that overlaps with both types of damage.

Fowler: Can the lack of killing at 0°C, the efficient killing at 37°C and the reverse dose rate effect be explained by invoking misrepair – or is this too vague a concept?